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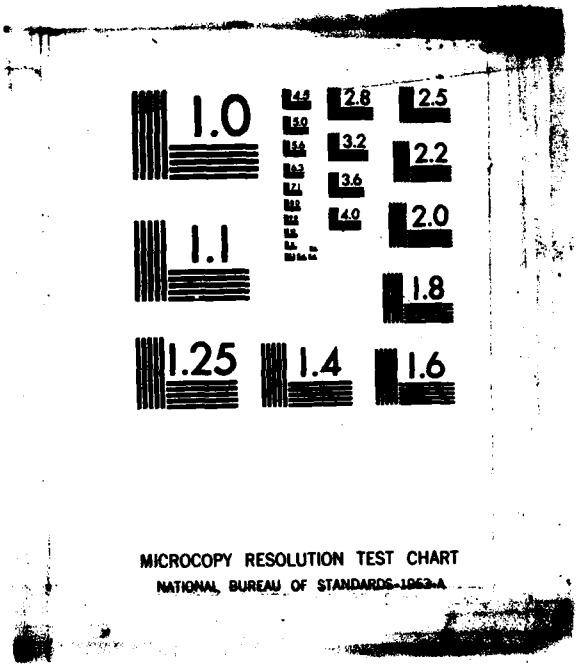
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CHEMICAL IONIZATION MASS SPECTROMETRY: NEW METHODOLOGY

FINAL REPORT

DONALD F. HUNT

JANUARY 8, 1983

U. S. ARMY RESEARCH OFFICE

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The view, opinions, and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy, or decision, unless so designated by other documentation

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

tandem mass spectrometry; peptide sequence analysis; ion-molecule; negative ions CI (chemical ionization); nitropolynuclear aromatic hydrocarbons; carboxylic acids collision activated dissociation; sulfur compounds

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

The goal of this research program was to develop new methodology for the detection and characterization of organic molecules by mass spectrometry. Specific areas addressed included; gas phase ion molecule isotope exchange reactions for counting hydrogen atoms in specific organic structural environments, optimization of tandem mass spectrometry for the direct analysis of complex mixtures of organics, sequence analysis of peptides in mixtures by the combination of collision activated dissociation and tandem mass spectrometry, fragmentation mechanisms of negative ions, and

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construction of a tandem quadrupole mass spectrometer with improved sensitivity for operation in the FAB ionization mode in the mass range, 1,000 - 2,000 amu.

A. Statement of the Problems Studied:

The goal of this research program was to develop new methodology for the detection and characterization of organic molecules by mass spectrometry.

Specific areas addressed included:

1. Gas phase ion-molecule isotope exchange reactions for counting hydrogen atoms in specific organic structural environments.
2. Optimization of tandem mass spectrometry for the direct analysis of complex mixtures of organics
3. Sequence analysis of peptides in mixtures by the combination of collision activated dissociation and tandem mass spectrometry
4. Fragmentation mechanisms of negative ions
5. Construction of a tandem quadrupole mass spectrometer with improved sensitivity in the mass range 1,000 - 2,000 amu.

B. Summary of the Most Important Results

1. Gas phase ion molecule isotope exhange reactions for counting hydrogen atoms in specific organic structural environments.

Hydrogens attached to aromatic rings, to carbons adjacent to aromatic rings, and to carbons alpha to carbonyl groups are readily exchanged for deuterium under CI conditions when deuterium labeled reagent gases are employed. The extent of deuterium incorporation decreases as the proton affinity between sample and reagent gas molecules increases and is temperature dependent as well. Under negative ion CI conditions, exchange of hydrogen for deuterium occurs at carbon adjacent to an aromatic ring in deuterium oxide, at carbon adjacent to a simple carbonyl group in deuteroethanol, and at the central carbon of a beta dicarbonyl group in deuterotrifluoroethanol. A mechanism for this process has been presented.

2. Optimization of tandem mass spectrometry for the direct analysis of complex mixtures of organics.

We have shown that the combination of chemical ionization, collision activated dissociation and tandem quadrupole mass spectrometry can be used for the direct analysis of complex mixtures for organics at the 10-100 ppb level. More than 100 carboxylic acids can be detected in urine using the above approach. Total sample preparation, instrument, and data analysis time per sample is about 15 minutes. All extraction, derivatization, and lengthy gas chromatographic separation procedures are obviated by the tandem mass

spectrometry approach.

Rapid qualitative screening of nitropolynuclear aromatic hydrocarbons in diesel particulates can also be accomplished using the triple quadrupole mass spectrometer operated in the constant neutral loss mode. This approach afforded excellent values for the quantitation of mutagenic nitropyrenes.

In another study positive ion CI, collision activated dissociation, and neutral loss scans for SH, (33 amu) on the tandem quadrupole instrument were employed for the selective detection of aromatic sulfur compounds in crude petroleum. Total time per sample for this analysis was under 20 min. (As part of a research effort sponsored by the USEPA we have developed a comprehensive scheme for the analysis of organics by functional group using neutral loss and parent ion scans on the tandem quadrupole instrument, D.F. Hunt, J. Shabanowitz, T.M. Harvey and M. Coates, J. Chromatogr 1983). 271, 97-105

3. Sequence analysis of peptides in mixtures by the combination of collision activated dissociation and tandem mass spectrometry.

Mass spectrometry is now playing an increasingly important role in the sequence analysis of proteins. Highly specific enzymes are employed to degrade the protein to a collection of small oligopeptides. These are then purified by some form of chromatography, derivatized, and then analyzed by conventional mass spectrometric techniques. In our early work we demonstrated that the time consuming purification of the oligopeptide mixtures could be obviated by using the tandem mass spectrometric approach to sequence permethylated peptides directly in complex mixture.

Following the development of fast atom bombardment, a new ionization method that eliminated the need to derivatize the oligopeptides, we showed that underivatized oligopeptides could also be sequenced by the tandem mass spectrometry approach. Unfortunately this approach required a sample level of at least 10-50 nmoles and was severely limited by the mass range (1,000 amu) of the first triple quadrupole instrument. Improvements in this methodolgy are described below.

4. Fragmentation mechanisms of negative ions

Negative ion CI with the hydroxyl anion as the reactant ion is ideally suited for the analysis of mixtures by tandem mass spectrometry because most compounds afford a single ion, M-H-, under these conditions. Detailed understanding of the fragmentation pathways open to these M-H- species under collision activated dissociation conditions is required if this process is to be used for structural characterization of organics. Our findings indicate that ketone M-H- ions undergo γ-hydrogen rearrangements with loss of olefin and retro Diels-Alder reactions. Sugars lose water and undergo retro aldol condensations, carboxylic acids lose carbon dioxide, and phenolic anions suffer electron detachment. Data accumulated in this study is too great to detail here in this report.

5. Construction of a tandem quadrupole mass spectrometer with improved sensitivity in the mass range 1,000 - 2,000 amu.

We have recently upgraded our home built triple quadrupole to a mass range of 2,000 amu using Model 4600 quadrupole rods from the Finnigan Corporation. Sensitivity in the FAB ionization mode on the earlier instrument was limited by high background noise at the electron multiplier. The source of this noise was excited neutrals produced in the discharge used to generate the energetic beam of charged or neutral Xe projectiles. The earlier instrument was also incapable of generating high yields of fragment ions in the collision activated dissociation process on molecules in the 900-1000 amu range.

To optimize the performance of the new triple quadrupole instrument in the FAB mode, we have moved the electron multiplier off axis, increased the conversion dynode voltage, placed an elbow in the ionization gage tubing, changed to 2 mm diameter gold plated probes floating at low potential (10-20 V), employed a Cs+ ion gun, and switched to thioglycerol as the liquid matrix. As a result of these modifications we have now improved the sensitivity of our instrument at high mass (1,000 - 2,000 amu) by more than a factor of 100. FAB spectra of polypeptides in this mass range can now be recorded routinely at the 50-100 pmole level. Complete structural information from CAD spectra can be obtained at the 300-1000 pmole level. (This is really an exciting development!)

We have used the above methodology to determine the amino acid sequence of an immunologically distinct delta hemolysin from a canine strain of Staphylococcus Aureus and to assign the position of phosphoseryl residues in phosphopeptides derived from bovine caseins.

Work is presently underway to build a tandem quadrupole Fourier transform ion cyclotron resonance mass spectrometer having a mass range of 10,000 amu and a detection level for large oligopeptides in the subpicomole range.

C. List of Publications

1. Gas Phase Ion-Molecule Isotope Exchange Reactions: Methodology for Counting Hydrogen Atoms in Specific Organic Structural Environments by Chemical Ionization Mass Spectrometry, D.F. Hunt and S.K. Sethi, J. Amer. Chem. Soc., 102, 6953-6963 (1980).

2. Collision Activated Decompositions of Negative Ions in Mixture Analysis with a Triple Quadrupole Mass Spectrometer, D.F. Hunt, J. Shabanowitz, and A. Giordani, Anal. Chem. 52, 386-390 (1980)

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13. New Ionization Techniques in Mass Spectrometry, D.F. Hunt, Int. J. Mass Spectrom. Ion Phys., 45, 111-123 (1982).

14. Ionization Techniques , D.F. Hunt, In "Tandem Mass Spectrometry", F.W. McLafferty, Ed., Wiley-Interscience, New York pp. xx - xx, 1983.

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16. Mass Spectral Analysis of the Primary Structure of Peptides: Assignment of the Position of Phosphoseryl Residues in Phosphopeptides Derived from Bovine Caseins, D.F. Hunt, D.W. West, and H.R. Morris, Biochem. J., (submitted)

D. List of Participating Scientific Personnel

Graduate Students:

1) William Bone Ph.D. May 1983 (employed at Walter Reed Army Medical Research Center)

2) Joseph Marasco Ph. D. August 1983 (employed as a postdoctoral research associate at the Walter Reed Army Medical Res. Center)

3) Anne Giordani Ph. D. May 1982

4) Jeffrey Shabanowitz Ph. D May 1983

5) Mary Sisak Ph. D. August 1981

6) Mildred Coates M.S. August 1982

7) Dennis Shaw

8) George Parker

Postdoctoral Students

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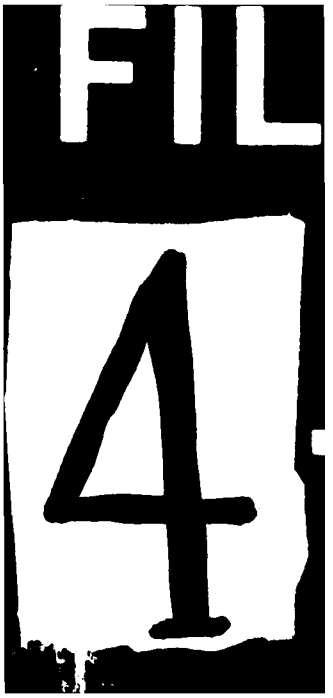
2) John Ballard

3) T. Michael Harvey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